Amendments to the Drawings

The attached sheets of drawings include changes to FIGS. 2, 4, 6, 14, 23B and 24B. These sheets, which include FIGS. 2, 4, 6, 14, 23A, 23B, 24A and 24B replace the original sheets including FIGS. 2, 4, 6, 14, 23A, 23B, 24A and 24B. In FIG. 2 reference number 52 has been deleted. In FIG. 4 reference number 24 has been changed to 26 and reference number 52 has been deleted. In FIG. 6 reference number 52 has been deleted and reference number 46 has been changed to 86. In FIG. 14 reference number 66A has been changed to 66. In FIG. 23B reference number 170 has been deleted. In FIG. 24B reference number 170A has been deleted.

Attachments: Replacement sheets (6)
Annotated sheets showing changes (6)

REMARKS/ARGUMENTS

The Office Action

In the above-mentioned Office Action, claims 322 and 385 were objected to due to various informalities; claims 322-429 were rejected as being indefinite; claims 322, 328-335, 342-345, 347-350, 352, 353, 385, 391-398, 405, 406, 408, 411, 413-415, 421-424, 428, and 429 were rejected as being unpatentable over U.S. Patent 5,637,458 (Frankel et al.) in view of U.S. Patent 6,375,901 (Robotti et al.); claims 323-327, 338-341, 351, 386-390, 401-404, 412, 416-420, and 425-427 were rejected as being unpatentable over Frankel, et al. in view of Robotti, et al. as applied to claims 322, 354, 385, and 415, and further in view of Heegaard et al. (*Journal of Chromatography B*, Sept. 11, 1998, Vol. 715, pp 29-54); claims 336 and 399 were rejected as being unpatentable over Frankel, et al. in view of Robotti, et al. as applied to claims 322 and 385, and further in view of U.S. Application Publication No. 2003/0134416 (Yamanishi et al.); claims 337 and 400 were rejected as being unpatentable over Frankel, et al. in view of Robotti, et al. as applied to claims 322 and 385, and further in view of U.S. Application Publication No. 2002/0115201 (Barenburg et al.); claims 346 and 407 were rejected as being unpatentable over Frankel et al. in view of Robotti et al. as applied to claims 322 and 385, and further in view of Heegaard et al. and U.S. Patent 5,246,577 (Fuchs et al.); claims 354, 361-367, 374-376, 378-381, 383, and 384 were rejected as being unpatentable over Frankel et al. in view of Robotti et al. and U.S. Patent 5,582,705 (Yeung et al.); claims 355-360, 370-373, and 382 were rejected as being unpatentable over Frankel et al. in view of Robotti et al. and Yeung et al. as applied to claim 354, and further in view of Heegaard et al.; claim 368 was rejected as being unpatentable over Frankel et al. in view of Robotti et al. and Yeung et al. as applied to claim 354, and further in view of Yamanishi et al.; claim 366 was rejected as being unpatentable over Frankel et al. in view of Robotti et al. and Yeung et al. as applied to claim 354, and further in view of Barenburg et al.; and claim 377 was rejected as being unpatentable over Frankel et al. in view of Robotti et al. and Yeung et al. as applied to claim 354, and further in view of Heegaard et al., and further in view of Fuchs et al. The

following claims were rejected under the judicially-created doctrine of obviousness-type double patenting as being unpatentable over claims 1-28 of U.S. Patent 6,406,604 on the following grounds: claims 322-326, 328, 330, 334, 335, 342, 347, 349-350, 352, 353, 385-389, 391, 392, 394, 397, 398, 406, 408-411, 413-419, 422, 428, and 429 in view of Robotti et al.; claims 327, 338-341, 351, 390, 401-404, 412, 416-420, and 425-427 in view of Robotti et al. as applied to claims 323, 385, and 415, and further in view of Heegaard et al.; claims 329, 331, 332, 343-345, 393, 395, 405, 423, and 424 in view of Robotti et al. as applied to claims 323, 385, and 415 above, and further in view of Frankel et al.; claims 336 and 399 in view of Robotti et at. as applied to claims 323 and 385, and further in view of Yamanishi et al.; claims 337 and 400 in view of Robotti et al. as applied to claims 323 and 385 above, and further in view of Barenburg et al.; claims 346 and 407 in view of Robotti et al. as applied to claims 323 and 385, and further in view of Heegaard et al. and Fuchs et al.; claims 354, 356-359, 361, 363, 366, 367, 378-381, 383, and 384 in view of Robotti et al. and Yeung et al.; claims 355, 360, 370-373, and 382 in view of Robotti et al. and Yeung et al. as applied to claim 354 above, and further in view of Heegaard et al.; claim 368 in view of Robotti et al. and Yeung et al. as applied to claim 354 above, and further in view of Yamanishi et al.; claim 369 in view of Robotti et al. and Yeung et al. as applied to claim 354, and further in view of Barenburg et al.; and claim 377 in view of Robotti et al. and Yeung et al. as applied to claim 354 above, and further in view of Heegaard et al. and Fuchs et al. The following claims were provisionally rejected under the judicially-created doctrine of obviousness-type double patenting as being unpatentable over claims 36-77 of copending Application No. 10/821,328 on the following grounds: claims 322-328, 331, 334, 335, 339-350, 352-361, 364, 366, 367, 371-381, 383-392, 395, 397, 398, 402-411, 413-420, 423, and 425-429 in view of Robotti et al.; claims 329, 330, 332, 333, 362, 363, 365, 393, 394, 396, 421, 422, and 424 in view of Robotti et al. as applied to claims 322, 354, 385, and 415, and further in view of Frankel et al.; claims 336, 368, and 399 in view of Robotti et al. as applied to claims 322, 354, and 385, and further in view of Yamanishi et al.; claims 337, 369, and 400 in view of Robotti et al. as applied to claims 322, 354, and 385, and further in view of Barenburg et al.; and claims 338, 351, 370, 382, 401, and 412 in view of

Robotti et al. as applied to claims 322, 354, and 385, and further in view of Heegaard et al.

The drawings were objected to as failing to comply with 37 CFR 1.84(p)(5).

In response to the Office Action, claims 322-429 are cancelled without prejudice or disclaimer and new claims 430-608 have been added; paragraphs 0092, 0123, 0124, 0141 and 0142 of the specification have been amended, and drawing FIGS. 2, 4, 6, 14, 23B and 24B have been amended.

Sequence of Events of the Present Invention

Set forth below is an exemplary sequence of operational events according to one or more aspects of the present invention.

1. A transport tube with an inlet and outlet port capable of carrying from the inlet first a sample, then one or more cleaning buffers, and in addition when desired a chromophoric or tagging substance. The valves of the transport passage are open, and the valves of the separation passages are closed.

2. The sample carrying the affinity target (or substance of interest) will bind to one or more affinity ligands immobilized in the matrix of the analyte concentrator, which has a unique and advantageous staggered configuration. (*See* "Staggered Configuration of the Present Invention" section below.) Since the sample may contain many affinity targets (or substances of interest), one or more affinity ligands in each of the several analyte concentrators located at the intersections of the transport passage and the various separation passages are immobilized.

3. Once the affinity target is captured by the immobilized affinity ligand(s) (in one or more of the analyte concentrators), a washing buffer can be passed through the transport passage to remove excess amounts of sample and unwanted material. If desired, a chromophoric or tagging solution can also be transported in the transport passage to bind the affinity target reversibly retained by the immobilized affinity ligand.

4. Another cleaning buffer can be added to the transport passage to remove an excess amount of chromophoric solution.

5. Now, the valves of the transport passage are closed and the valves of the separation passages are open.

6. The separation passages can also have inlet and outlet ports. The transport passage is of the same inner diameter or preferentially larger that the inner diameter of the separation capillaries. The larger inner diameter can facilitate the speed of the transport of the samples, cleaning buffers, and chromophoric solutions.

7. A separation buffer can be added to the separation passage, if the separation buffer is mild and does not affect the binding between the immobilized affinity ligand and the affinity target. However, if the buffer interferes with the binding, an auxiliary passage may be provided to maintain the binding between the immobilized affinity ligand and the affinity target under optimal conditions.

8. Once the system is clean and the affinity target is captured under the appropriate orientation and optimal conditions, a small volume or plug of an elution buffer can be added to the separation passage to liberate the affinity targets from the immobilized affinity ligands.

9. The process of separation starts towards the one or various detectors for identification and characterization purposes.

10. Another analyte concentrator can be positioned in the separation passage to bind a chromophoric substance for further tagging a separated affinity target.

11. The samples can be separated in individual separation passages with the need of one individual detector (or a detector that can have a mechanism that moves to the second separation passage to monitor another affinity target in a second separation passage). Alternatively, the separation passages can be merged to form a single exit outlet and using a single detector.

12. Several detectors can be used simultaneously for either the individual or the merged passage systems.

13. The passages can empty into a waste container or a reservoir that collects fractions for further use.

Staggered Configuration of the Present Invention

All of the claims in this application include, *inter alia*, the novel "staggered" configuration feature of the present invention. The staggered configuration for the intersection of the transport passage with a separation passage is shown, for example,

in FIG. 11b, which can be contrasted with the non-staggered passage as shown in FIG. 11a. The staggered configuration is also shown in FIGS. 18, 19, 22, 28a and 28b. Paragraph [00355] discusses the staggered construction as illustrated in FIGS. 28a and 28b.

The analyte concentrator is designed in a staggered configuration in the above-mentioned FIGS. 18, 19, 22, 28a and 28b to allow the passage of extremely large volumes of fluids (e.g., microliters and milliliters). The staggered configuration has a very large surface area to permit the maximum capturing and concentration detection of any type of analyte, such as small molecular substances and biomolecules.

Paragraph [00313] discloses how the transport capillary can be staggered from a separation capillary to form a staggered concentrated area. The area is elongated which allows additional matrix-like assemblies to be incorporated therein to attach a desired affinity ligand to capture a desired target analyte from the sample solution. In addition, the sample solution takes additional time to pass through the elongated concentrated area, which allows the desired affinity ligand immobilized to the matrix-like assembly additional time to bind to the desired target analyte from the sample solution. The concentrated area may be surrounded by frits or porous end plates to retain the matrix-like assembly within the concentration area or it may contain a matrix-like assembly within the concentration area interconnected without the need for frits.

Paragraph [00319] mentions that an elongated concentration area, as disclosed in FIG. 11b, may be provided to expose the sample solution to the matrix-like assembly for a longer period of time and a longer surface area to capture larger amounts of desired analytes.

Paragraph [00339] discloses how the valves can be operated so that the sample solution passes through the staggered area as desired.

Thus, the present invention has a staggered configuration to immobilize affinity ligands, to enhance the surface area of binding, to create a microenvironment to facility optimization of binding utilizing optimal temperatures and micromixing, to even be able to derivatize (discussed in detail below) components at the analyte concentrator micro-reactor or using a second concentrator. Further, the analyte concentrator in the

staggered configuration not only is a concentrator, but can also be a micro-reactor allowing multiple enzymatic/chemical/ biochemical/cellular/and subcellular reactions to occur.

The staggered configuration of the present invention can allow chemical synthesis to occur in a microenvironment. The present invention can permit cellular and subcellular organelles to be trapped and allow the formation of endogenous metabolites or induced metabolites to a particular drug.

Further and perhaps more importantly, the present invention has the analyte concentrators at and within the staggered configuration. It further has valving surrounding the staggered configuration to control the flow of fluid from the transport passage through the staggered configuration and into the associated separation passage.

Even further, the device with the staggered configuration can be connected to another device containing a second staggered configuration with a second matrix-like assembly within the concentration area, in which one or more affinity ligands can be immobilized to capture additional target analytes. This unique arrangement permits the system to be a high-throughput and multi-dimensional system assaying hundreds of samples in a short period of time, in a continuous and sequential manner, one channel or capillary functioning at one time if one detector is used, or multiple channels or capillaries functioning simultaneously if several detectors are used; in particular, affinity target(s) found at low abundance in biological materials or chemical samples. Further, multiple enzymatic/chemical/ biochemical/cellular/and subcellular reactions can be carried out in a few hours.

The separation passages associated with the staggered configurations have the capability of fraction collection, permitting the collection of extracted, concentrated and purer samples from biological materials or chemical sample solutions.

The staggered configuration with the valves does not permit entrance of raw samples into the separation passage. Only the transport passage made of PEEK material transports the sample. This has the advantage that the clean separation capillary can be re-used many times.

On-Line Derivatization of the Present Invention

According to one aspect of the present invention, on-line derivatization is used. In other words, the analyte concentrator can capture a target analyte present in a simple or complex mixture via an immobilized affinity ligand. The rest of the components of the matrix can be washed away using one or more buffers. Once the area of the analyte concentrator is clean, a chromophore can be added to the analyte concentrator through the transport tube to permit derivatization of the bound target analyte. The excess amount of chromophore is cleaned away and then an elution process is started using a small plug of an elution buffer through the inlet of the separation capillary or passage, followed by a separation buffer to separate the derivatized substance(s) eluted from the analyte concentrator.

Auxiliary Passage of the Present Invention

Many of the dependent claims now pending include the novel "auxiliary passage" feature of the present invention. The auxiliary passages are shown for example in FIG. 17 at 122, 124, 126. They are further shown in FIG. 18. FIG. 19 shows an enlarged view of one of the passages 130.

Referring to paragraph [00102], the auxiliary passage is coupled to the separation passage downstream from the analyte concentrator to provide separation buffer to the separation passage away from the concentrator.

Referring to paragraph [00331], FIG. 17 illustrates that the new separation buffer solution can be added by auxiliary passages 122, 124 and 126 downstream from the concentrators in order to preserve the integrity of the antibody or any other immobilized affinity ligands. In certain applications, the analytes under study may require for optimal separation, separation buffer solution that may adversely affect the activity of the intact antibody, antibody fragment, lectin, enzyme or any affinity ligands affected by certain compounds present in the separation buffer. In other words, certain separation buffer solutions may adversely affect the binding property of the immobilized affinity ligands in the concentrator so that the affinity ligands can be damaged and may not be used again. Also, the analytes may not be retained by the immobilized affinity ligands.

With the auxiliary passages 122, 124 and 126, the separation buffer solution may be introduced into the separation capillaries using the cups 128, 130 and 132, for example. This allows the separation buffer solution to flow towards the detecting zone so that there is no interaction whatsoever between the separation buffer solution and the antibodies in the concentrators. For example, the separation of an analyte may require the presence of organic solvents or other additives in the separation buffer solution such as urea, certain detergents, etc. If such separation buffer solutions pass through the concentrators so that the separation solution interacts with the antibodies in the concentrators, the separation buffer solution may disrupt the binding process between the analyte and the antibody during the conditioning process of the capillary and/or destroy the quality of the immobilized antibody in an irreversible manner. Such adverse effect on the antibody may destroy the integrity of the binding capacity of the antibody so that it may not bind to the analyte and/or may not be used again. To substantially prevent such adverse effect on the antibody, the antibody in the concentrator is isolated from such separation buffer solution to protect the immobilized antibody, antibody fragments or other affinity elements.

Paragraph [00332] continues by saying that in addition, the binding and separation conditions of the desired analyte may require different optimization conditions. Paragraphs [00332]-[00335] further describe the auxiliary passages and the variations and operations.

The auxiliary passages are connected to the separation passages and can include either two or three valves for introducing a new separation buffer that will not interfere with the microenvironment of the analyte concentrator-microreactors.

Furthermore, it is stated in the specification that "Fig. 17 is a cross-sectional view of a region of the cartridge of Fig. 16 containing filter paper for capturing analyte." The system of the present invention does not use filter papers as analyte concentrators. Rather, it uses more inert, reliable and reproducible kinds of materials able to resist aqueous and non-aqueous environments.

The first section or portion of the separation capillary (starting in the inlet port and ending in the analyte concentrator) has the optimized buffer conditions, including

temperature, to maintain the affinity target attached in a non-reversible form to the immobilized affinity ligand. The second section or portion of the separation capillary (starting in the analyte concentrator and ending in the outlet port) has the optimized buffer conditions, including temperature and additives, to separate the target analyte(s) with the highest resolution and highest sensitivity. In order to elute the non-reversible bound affinity target, a plug of an elution buffer is introduced into the first section of the separation capillary at the inlet port, followed by the same separation buffer that was introduced through the auxiliary passage into the second section of the separation passage, continuing the separation process of the affinity target(s) through the detector system(s) until the end of the outlet port.

Claims are Patentable over Frankel v. Robotti

The reasons why Frankel and Robotti do not teach individually or in combination the inventions of the present claims and why their teachings cannot be combined are set forth in the paragraphs below.

Teaching or Suggestion Can NOT Be Based on Applicant's Disclosure

Contrary to the statement in the Office Action (see the last sentence of the paragraph of page 14 in the Office Action beginning with "Furthermore"), the teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art and not based on applicant's disclosure. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991). MPEP 706.02(j) and 2143. Additionally, "[i]t is impermissible within the framework of section 103 to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one skilled in the art." *In re* Wesslau, 147 USPQ 391, 393 (CCPA 1965); *see also In re* Mercer, 185 USPQ 774, 778 (CCPA 1975), and *In re Hedges*, 228 USPQ 685, 687 (Fed. Cir. 1986). The Section 103 rejections employ this impermissible "picking and choosing," Applicant submits.

Frankel is Not Relevant

The main objective of Frankel is to separate species of organic molecules, and

for detecting and quantifying the separated molecular species with a continuous wave laser through which the separated organic molecular species is conveyed.

Frankel says that chemical and biochemical separation technologies have been extensively developed over the past 100 years (col. 1, lines 32-33), of which two of the most common separation technologies in current application are electrophoresis and chromatography (col. 1, lines 38-39). The Background of the Invention describes the various characteristics of electrophoresis and chromatography, including isoelectric focusing and capillary electrophoresis. Also described are "two-dimensional electrophoresis" and "fingerprinting" (col. 2, lines 60-62).

The main goal of Frankel is to "provide a system of quantitative molecular detection that offers detection sensitivity below the 0.1 picomolar range, and eliminate the use of reporter molecules" (col. 6, lines 21-23).

The Frankel system is described as an "apparatus for the identification of a molecular species in a fluid comprising a first laser having a plurality of reflective optical layers and a laser material disposed therebetween, and having photonic energy there-though for interaction therewith to produce a coherent output" (col. 6, lines 37-42).

The so-called "reporter" molecule is used for detection. In this method the molecule of interest is brought into contact with a reagent, which binds to the molecule and provides its own measurable signature (col. 5, lines 56-58). The reporter molecule is sometimes a radioactive "tag" attached to the molecule of interest (col. 5, line 66).

Frankel says that the most important detection system is the continuous wave laser detector described in this patent. The use of the reporter molecules is not so important and must be eliminated. For example, Frankel says: "In accordance with yet another aspect of the invention an affinity coating is disposed on an interior surface of the sample chamber for binding with the molecular species" (col. 6, lines 60-63) and "In accordance with still another aspect of the investigation a microlens is disposed in the first laser for converging the photonic energy onto a small region of the sample chamber, the affinity coating being disposed substantially on the region" (col. 6, lines 64-68).

When Frankel refers to "affinity coating disposed on an interior surface", there is

no description if the affinity molecules are bound covalently or adsorbed in the surface non-covalent by physical and/or chemical attractions. Because of the nature of the binding there will be an optimal orientation of the affinity molecule to capture the antigen, hapten, or other corresponding affinity molecule. (Analogously, if the baseball catcher's glove is not in position to catch the baseball, he will miss the ball.) Orientation of the immobilized affinity molecule enhances the capture of the corresponding affinity molecule of interest. There will be more surface area to enhance sensitivity and quantification; it will permit reusable assays, active readout, and good end point detection. Otherwise, even if the detector is a state-of-the-art detector, and if there are only a few molecules to detect, the quantification will be inferior to the quality described in the present application: "a staggered analyte concentrator with the largest surface area, and 100% of orientation of the immobilized affinity ligands will capture the most of the molecules of interest". If the immobilized affinity molecules are not oriented, as few as five molecules or less in a certain surface area may be captured. With the staggered analyte concentrator of the present invention (and with oriented immobilized affinity capture molecules) 10,000 or more of the molecules of interest may be captured in the same surface area.

Although the title of the Frankel patent is "Apparatus and Method for the Detection and Assay of Organic Molecules", it states: "[t]he means for separating comprises a molecular sieve comprising a plurality of nanostructures, the nanostructures comprising spaced apart post defining channels therebetween for passage therethrough of molecules." (Col. 7, lines 5-8.)

The organic substance that Frankel refers to most often is the separation of chromosomes (very large complexes of molecules). The molecular sieve that it uses to separate chromosomes has a pore size of 0.5 – 0.02 micrometers (col. 7, line 11). The molecular sieving separation is repeated again at col. 8, lines 5-11: "The invention provides an integrated system for organic separation and detection. It consists of planar microstructures for separation coupled to a unique, miniaturized, laser based detection system. The planar microstructure is an electrophoretic channel containing a two-dimensional nanostructure which acts as a molecular sieve. Typical pore sizes

range from 0.5 – 0.02 micrometers." There is another description of molecular sieving in planar microstructures at col. 8, lines 49-54: "Lithographically defined solid state sieves also differ from gel based sieves in that the provide sieving in only one of the two axes orthogonal to the direction of flow. This difference in sieving action is especially pronounced in the case of rod shaped molecules undergoing separation."

At col. 7, lines 39-46, Frankel mentions a plurality branch points and microvalves: "In accordance with still another aspect of the invention the network comprises: a branching network of electroosmotic channels, having a plurality of branch points, each the branch point being guarded by microvalve that is selected between an open position and a closed position; and means for controlling the microvalves; whereby the chromosome can be directed along a selected one of a first arm and a second arm of the branch point". At this point Frankel mentions a branching network, but he does not refer to multi-dimensional, fully-automated control of the separation of hundreds or thousands of selective substances present in complex matrices, use after capture and release of the trapped antigens or hapten, or other affinity ligands, or any of the nine modes of capillary electrophoresis, including but not restricted to molecular sieving.

Robotti Is Not Relevant

The main objective of Robotti is to create a microvalve for use in micro-fluid devices that is different from earlier microvalves. Most valves or microvalves have been designed with materials derived from metal, plastic, polymeric materials, ceramic, glass, or the like. The actuation of the valves/microvalves is mechanical.

Robotti, on the other hand, is made of a "reversible gel", a substance that can be polymerized and become a blocking surface, preventing the passage of fluid, when it is in a "solid state", and can permit the passage of the fluid when it is in a "soluble state". "Any phase reversible material may be employed, so long as the material changes in phase in response to an applied stimulus in a manner sufficient to modulate its fluid permeability, i.e., the ability of fluid to flow through the material." (*See* col. 4, lines 31-34).

Heating element 15, which is shown in FIGS. 1, 2, and 3, is like a resister that when heated converts the polymer from a solid state to a soluble state. The applied

stimuli of interest include temperature, pH, electrical current, light, magnetic field, etc. (*See* col. 4, lines 39–40).

The shape of the micro-compartments is described at col. 3, lines 27-33, and the configuration of the micro-channels is described at col. 3, lines 40-49.

Robotti Does Not Disclose the "Staggered Configuration"

FIG. 4 of Robotti describes the purpose of every channel, and FIG. 5 is only a variation of FIG. 4. Channels 31 and 33 are entry ports, meaning introduction of samples. Ports 35 and 37 are exit ports, meaning exit of the separated analytes, buffers, etc. to a kind of a waste container. Channels 32 and 34 are the main Intersecting microchannels, where separation apparently occurs. All other numbers: 36, 38, 39, 40, and 50 are the reversible microvalves that control the sample introduction into the flow channels.

In col. 10, lines 23 to 58, it is described the uses or functions of the microchip with the valves. Some of the functions include sample preparation, separation, and chemical synthesis applications. Robotti also describes the use of kits in electrophoretic and chromatography applications.

However, Robotti does not describe any process of on-line preconcentration of samples containing substances at low abundance, introduced into the capillary or microchannel at very large volumes such microliters or milliliters. Although it mention the use of sample preparation, it does not mention the use of affinity elements.

Robotti focuses on a single channel. In contrast, the present invention can be a high-throughput multi-dimensional device capable of many more uses than that described in Robotti.

The system of FIGS. 4 and 5 of Robotti is a discontinuous system. In other words, the entry port is a hole connected the exit port (another hole) through a channel. The user adds a certain volume of sample in the entry port and that sample volume is sufficient for only one assay. In contradistinction, the system of the present invention, according to one aspect thereof, is a continuous system. In other words, the entry port of the staggered configuration of the present invention is connected to the exit port of the device through a capillary or channel and the transport passage delivers samples to

a plurality of passages (channels or capillaries) connected mainly in a parallel arrangement. This unique arrangement permits the system to be a high-throughput and multi-dimensional system assaying hundreds of samples in a short period of time, on a continuous and sequential manner, one channel or capillary functioning at one time if one detector is used, or multiple channels or capillaries functioning simultaneously if several detectors are used.

Frankel v. Robotti Does Not Teach the Present Invention

Robotti focuses primarily on a valve made of a reversible gel, which is capable of changing from a solid state to a soluble state.

Frankel focuses primarily on the enhancement of sensitivity through use of a continuous wave laser.

Although both patents referred in a low importance to other aspects of capillary electrophoresis, neither patent stresses:

- Enhancement of sensitivity by immobilized affinity ligands.
- Enhancement of sensitivity by tagging substances (in fact, Frankel option is to eliminate them).
- The analyte concentrator as a device for enzymatic reactions, antigen-antibody reactions, other affinity ligand-receptor reactions, etc.
- An analyte concentration as a chamber having features for mixing and providing the appropriate temperature and other conditions necessary for optimization.
- An analyte concentrator that contains a "staggered" or Zeta configuration to increase the surface area for capturing more antigen, or hapten, or substances of related affinities. This method enhances sensitivity by several thousand-fold.
- An analyte concentrator in which molecules are covalently immobilized by the most appropriate orientation of the molecule, in order to capture the most of the corresponding affinity ligands.

- An analyte concentrator having an auxiliary passage connected to the separation passage, and intersected by microvalves, in order to provide alternative buffers to separate compounds that needed drastic buffers. The immobilized affinity ligands to the analyte concentrator have better chances of functioning under very mild conditions. Therefore, the immobilized affinity ligand can not change their structural properties and hence be able to be re-used several times.

- The analyte concentrator having a "staggered configuration" permitting the attachment of several "staggered configurations" in parallel to create a multi-capillary, multi-channel, and multi-dimensional apparatus capable of high-throughput capabilities. A single multi-dimensional immunoaffinity capillary apparatus having all features described above, may well assay several hundred samples a day, with possibilities of several thousand samples to be assayed.

- The outlet capillaries can exit independently, or merge two or more capillaries creating a single outlet exit capillary with capabilities of fraction collection of extracted, concentrated and purer samples than those found in the original simple or complex chemical or biological mixtures.

FIG. 1 and its description of Frankel are not the same as the invention of the present claims. The Office Action apparently evaluates certain aspects of chromatography and capillary electrophoresis separation, with certain unique detection systems, some valves and molecular sieving separation, and some affinity ligands in Frankel and is adding to this scheme the valves described in Robotti made of "reversible gels" that can convert from a "solid state" to a "soluble state", and comes to the conclusion that the present invention and its many claimed features are thereby rendered obvious. This is clearly an improper analysis and conclusion.

Franklin Does Not Disclose an "Auxiliary Passage"

The Office Action says that Frankel "teaches an auxiliary passage (1325 in Fig. 13) coupled to at least one of the separation passages downstream of the analyte concentrator to provide a fluid to the separation passage away from the analyte

concentrator and valve means (1312 in Fig. 13) for controlling flow out of the auxiliary passage (col. 20, lines 21-35)." Applicant respectfully disagrees as discussed below.

Passage 1325 as shown in Frankel FIG. 13 is an exit channel in which all eluted pieces of DNA are now going in the appropriate direction to the detector 1320. That is why it is called a "readout column", because the separated samples are ready to be read (or monitored) by the detector. This is basically the equivalent in the present application, in which the separation passage, having a linear configuration or any other configuration, is positioned on or at the detector, so that the separated compounds can be monitored by the detector. There is nothing auxiliary; it is simply the path to reach the detector. The only difference in the design with respect to the present invention is that the so-called "readout column" is bent so it can be used with a single detector, because all exits converge into the single capillary. Otherwise, it would need several detectors, one for each channel. In the present application, three or more separation passages can merge into a single exit passage to use a single detector. The actuated microvalve 1312 brings the separated and detected DNA substances to the sink or waste reservoir 1335.

The concept of the "auxiliary passage" in the present application is an optional design to apply more drastic buffers to the separation capillary or channel. It is not mandatory like in Frankel, where the "readout column" is required to read the samples.

Heegaard "Analyte Concentrator" Would Not Be Substituted for Frankel "Analyte Concentrator"

"Affinity capillary electrophoresis: Important application areas and some recent developments" (Heegard, et al. *J Chromatogr B* 1998; 715: 29-54.), is co-authored by the present inventor, Dr. Guzman. One skilled in the art would not substitute a Heegaard "analyte concentrator" for Frankel's analyte concentrator for reasons set forth below.

Referring to Frankel FIG. 13, at reference numeral 1303 is the area of sample introduction into a linear and single straight channel. The sample (isolated fragments electrophoretically transported) goes through a series of DNA array of probe chambers. In the direction of the sample, there are no valves. As very clearly is explained in the

FIG, there is no staggered configuration of the channel or valves in the direction of the sample. Column 19, lines 55-58, says that the "channels are approximately 20 micrometers wide and 1 micrometer deep filled with lithographically fabricated, diamond shaped, solid nanostructures with 0.1 – 0.2 micrometer spacing to maintain chain elongation". These channels are used to switch or direct chains between multiple polymerase Chain Reaction amplification stations. The amplified chains may be then transported through further nanostructure bearing channels to chambers for further digestion, separation, and sequencing, or used directly for DNA probe hybridization (column 20, lines 5-10).

The sizes of these channels and "probe chambers" are probably similar to the previously described channels. The volumes in these microarrays may well be in the order of nanoliters (limiting the amount of sample introduced into the channel). There is no use of vacuum or pressure; there is no shaking, heating, or mixing in the "analyte concentrators" or "probe chambers". The samples are already concentrated, the array of probes are only used to capture them for cleaning process and for specifically capturing those that perfectly match the probes (complementary pieces of DNA). There is description of only capturing ("probe chambers"). There is no description of microreactions in these "probe chambers". The valves are located in the elution buffer area (1310 and 1315), termed there "denaturing reagents" (FIG. 13, 1302), and later, in the exit passages (1313 and 1313). There are no valves in between "analyte concentrators" or "probe chambers".

In the analyte concentrators of the present invention, the sample can be introduced in volumes of nanoliter, microliter, and milliliter quantities (there is no limitation in volumes). The sample may be introduced by electrophoretic migration (electrokinetic), vacuum, and/or pressure. The area of sample introduction is a staggered configuration, as discussed in detail above. Furthermore, in the linear area of the staggered configuration is located the "analyte concentrator–microreactor", performing functions of capturing (e.g., antibody-antigen) and of microreactions (e.g., enzyme-substrate, peptide synthesis). The concentrator may have affinity binding ligands bound directly to the inner wall of the capillary or channel, to various forms of

beads trapped in between frits, beads bound to each other and to the inner wall of the capillary without frits, or a network of microstructures that include sol-gel, monolithics, or other forms, without frits.

The passage of sample from the inlet reservoir through the outlet reservoir is accomplished by the "transport passage" (larger plastic tube, capillary or channel) intersect with each separation capillary or channel. In between each analyte concentrator – microreactor, at the intersection area four microvalves can be provided. The area of the analyte-microreactor can be subjected to direct or indirect shaking, heating, and mixing. The immobilized affinity binding ligands are not only limited to DNA probes, but to a wide range of affinity binding interactions, such as antibody-antigen, antibody-hapten, lectin-sugar, enzyme-substrate, aptamer-DNA, aptamer-peptide, aptamer-protein, protein-DNA, DNA-DNA, protein-protein, protein-sugar, protein-lipid, protein-small molecular weight compounds, etc.

At the beginning of the separation capillary, after the analyte concentrator-microreactor location, there can be an auxiliary tube providing a wide range of buffers, including "harsh buffers", to the separation capillary. (The auxiliary passage is discussed in detail above.) The valve connecting the analyte concentrator-microreactor to the separation capillary will prevent these "harsh buffers" from damaging the integrity of the affinity ligands immobilized to the analyte concentrator–microreactor area. The elongated configuration (staggered configuration) of the analyte concentrator permits a very large surface area of immobilized affinity ligands that enhances sensitivity or detector response for many chemicals, including those found in very small quantities in complex matrices.

After the complex sample containing a very large amount of substances is introduced through the transport capillary, and the selective number of target affinity compounds are captured by the immobilized affinity ligands, one can clean the excess amount of unwanted material, and add a chromophore that will bind "in situ" (on-line derivatization) to the captured affinity target. The eluted substance(s) will be detected by ultraviolet and/or fluorescence detection, increasing the sensitivity of the sample even further. Furthermore, in addition to performing analyte concentration or

microreaction, the analyte concentrator–microreactor can also perform chemical synthesis "in situ."

The outlet capillaries can exit independently, or merge two or more capillaries creating a single outlet exit capillary with capabilities of fraction collection of extracted, concentrated and purer samples than those found in the original simple or complex chemical or biological mixtures.

Conclusion

Accordingly, it is respectfully contended that all of the claims now pending are in condition for allowance. Issuance of the Notice of Allowance at an early date is thus in order.

If there are any remaining issues, Examiner Jung is encouraged to telephone the below-signed counsel for Applicant at (310) 785-5384 to seek to resolve them.

The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. 10-0440. Should such additional fees be associated with an extension of time, Applicant respectfully requests that this paper be considered a petition therefor.

Respectfully submitted,

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